Postharvest Biology and Technology 49 (2008) 283–286

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Methyl bromide fumigation of packed table grapes: Effect of shipping box on gas concentrations and phytotoxicity

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ARTICLE INFO

Article history:
Received 2 October 2007
Accepted 19 March 2008

Keywords:
Fumigation
Postharvest
Quarantine
Export
Grapes
Methyl bromide
TKV
EPS
Shipping box
Phytotoxicity
Sorption
C × T product

ABSTRACT

Current methyl bromide (MB) schedules for table grapes to control pests of quarantine significance are approved for fruit packed in Toyon Kraft Veneer (TKV) boxes. The question arose concerning equivalence of exposure to MB if an expanded polystyrene (EPS) box was used in lieu of the TKV box for table grapes being shipped to foreign countries. Fumigations of 'Crimson Seedless' table grapes packed in either EPS or TKV boxes were conducted for comparison of MB gas concentrations and fruit quality using Australian (AQIS) MB treatment schedules. Methyl bromide exposure expressed as the concentration × time (C × T) product ($g\,h\,m^{-3}$) was the same or higher for the EPS box compared to the TKV box. Methyl bromide gas concentrations were higher for EPS boxes at the start of the fumigation, but lower than TKV boxes at the end of the exposure period (2 h) due to a higher sorption rate of MB into the EPS Styrofoam® material. Evaluation of fruit quality showed internal browning, a typical characteristic of MB phytotoxicity, was absent or very low (<1.7% of the berries) in table grapes packed in both box types. No other injury attributable to exposure to MB was observed. Since C × T product exposure to MB was equivalent or even higher when EPS boxes were used, EPS boxes should be suitable for shipping table grapes to export markets. However, if the practice of determining exposure to MB continues to be based on readings of gas concentrations at the end of the 2-h exposure period, then data show that an increase in MB dose of $4\,g/m^3$ is suggested when the EPS box is used in lieu of TKV boxes for shipping table grapes to export markets.

1. Introduction

In shipping grapes to Australia the question has been raised as to whether or not the current schedule for grapes using methyl bromide (MB) will provide the same efficiency to control pests of concern to Australia by having a negative impact on MB gas concentrations if a different shipping box is used. Table grapes shipped to Australia are required to be packed in Toyon Kraft Veneer (TKV) boxes or corrugated plastic boxes, while cardboard cartons and expanded polystyrene (EPS) boxes are not permitted. At times, the grape industry in California would prefer to use the EPS shipping box, but there is earlier research showing increased sorption of MB by the EPS box compared to TKV boxes (Harris et al., 1984; Smilanick et al., 2000). Tests were conducted to establish MB concentrations over time when fumigating table grapes in TKV compared to EPS shipping boxes. The treatment schedules chosen were based on those required by the AQIS April 2004 Work Plan. Two test series were designed to examine possible differences in MB gas concentration, sorption, and concentration times time (C × T) product between the two box types. If the box type significantly modified in MB treatment parameters, a new treatment schedule could be devised to compensate for lower end concentrations and to ensure equivalency. Phytotoxicity evaluations of the treated and untreated grapes were made to determine any loss in fruit quality due to exposure to MB at the schedules tested.

2. Materials and methods

2.1. Fumigation

All tests were conducted in $0.242\,m^3$ steel chambers with air circulation fans. The chambers were housed inside a specially designed temperature controlled room and attached to an exhaust manifold. Treatment temperatures were accurately controlled and the fumigant safely exhausted from the chamber at the end of the exposure period. The chambers exceeded all standards for seal and tightness. Routine empty-chamber tests show no appreciable loss of fumigant due to leakage or sorption. The treatment schedules of concern and tested were those proposed by the Australian

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doi:10.1016/j.postharvbio.2008.03.008

Table 1
Series I comparisons: MB gas concentrations, sorption, and C × T products from fumigation of EPS or TKV boxes with treatment schedules per AQIS April 2004 work plan for Californian Table Grapes: 32 g/m^3 at 21 °C or 48 g/m^3 at 10 °C

Box type	Sample	MB gas concentration (g/m^3; mean ± S.D.) over time (min)				% Sorption	C × T product (g h m^{-3})
		Start (5)	30	60	120		
32 g/m^3 for 2 h at 21 °C							
EPS	Air	58.4 ± 4.1	36.8 ± 1.7	28.7 ± 1.1	19.4 ± 1.3	66.7 ± 1.3	62.7 ± 3.2
	Box	47.0 ± 2.6	36.6 ± 1.1	28.5 ± 0.8	19.0 ± 0.6	N/A	59.3 ± 2.0
TKV	Air	39.3 ± 0.9	31.3 ± 0.4	29.4 ± 0.4	27.0 ± 0.2	31.3 ± 1.4	59.7 ± 0.6
	Box	31.6 ± 5.7	31.0 ± 0.5	29.2 ± 0.3	26.8 ± 0.2	N/A	57.4 ± 1.8
$P_{0.05}$[a]		>0.0001	>0.0001	0.024	>0.0001	>0.0001	0.025
48 g/m^3 for 2 h at 10 °C							
EPS	Air	60.5 ± 2.3	52.2 ± 1.6	46.2 ± 1.3	37.9 ± 1.2	37.3 ± 1.2	92.6 ± 2.8
	Box	58.7 ± 1.7	51.9 ± 1.4	46.0 ± 1.1	37.9 ± 1.1	N/A	91.9 ± 2.3
TKV	Air	52.5 ± 3.1	47.5 ± 2.3	44.7 ± 2.1	42.0 ± 1.8	19.9 ± 1.7	89.4 ± 4.3
	Box	50.9 ± 3.3	47.0 ± 2.0	44.4 ± 2.0	41.7 ± 1.8	N/A	88.4 ± 4.1
$P_{0.05}$[a]		>0.0001	>0.0001	0.026	>0.0001	>0.0001	0.022

[a] ANOVA: differences between EPS and TKV boxes, within treatments, are not significant if $P > 0.05$ or significant if $P < 0.05$ (SAS, 2002–2003).

Quarantine and Inspection Service (AQIS) in their Work Plan for Californian Table Grapes (April 2004) for control of pests of quarantine significance to Australia. AQIS requires use of the TKV shipping box, but the California grape industry would prefer to use the EPS shipping box. Two treatment schedules were selected for testing: 32 g/m^3 at 21 °C for 2 h and 48 g/m^3 at 10 °C for 2 h. Exposure time for all tests was 2 h at normal atmospheric pressure (NAP) followed by a 4 h aeration period. Load factor (v/v) for each box type was made the same as closely as possible by pacing three boxes of table grapes in either EPS or TKV boxes inside each chamber representing either a 41% load or 44% load of 'Crimson seedless table grapes being fumigated. In Series I tests, table grapes in either EPS or TKV boxes were exposed to the both MB schedules: 32 g/m^3 for 2 h at 21 °C and 48 g/m^3 for 2 h at 10 °C to determine the degree of sorption of MB gas and calculate C × T products for each box type. Based on MB gas concentrations obtained at the end of the exposure period (2 h) in the Series I tests, MB doses were adjusted or modified accordingly for EPS boxes in Series II tests to try to match end readings for both box types. The boxes of test fruit were pre-conditioned overnight to the desired treatment temperature before being fumigated. MB gas concentrations were measured and recorded over time at the start (5 ± 2 min) of exposure, and at 30, 60, and 120 ± 2 min both inside and outside the boxes. C × T products were calculated using the method of Monro (1969). Following fumigation, the boxes of grapes were moved to cold storage at 1 °C. Series I tests were replicated four to five times and Series II tests six times. One-way analysis of variance (ANOVA; SAS Institute 2003) was used to analyze the concentration data ($P > 0.05$ not significant; $P \leq 0.05$ significant)

2.2. Phytotoxicity

After fumigation, the grapes were stored 3–4 weeks at 1 °C and then 2 d at 20 °C and their quality assessed by evaluation of cluster and rachis appearance, shatter, and internal browning of the berry. Nine clusters per package were examined, and from 2 to 18 packages were examined from each treatment. Series I berries were harvested in September and Series II berries in October from the same vineyard near Delano, CA. Control berries were harvested between Series I and II harvest dates. Overall cluster appearance and rachis appearance were recorded by a visual index, where 0 = perfect and 4 = unacceptable. Shatter and decay were recorded as the percentage of detached or decayed berries, respectively, within a cluster. Internal berry browning was determined by longitudinally slicing 10 berries per cluster into 3 portions, and placing the middle portion on a lighted box to determine if the flesh was brown colored.

3. Results

3.1. Fumigation

Load factor (v/v) is calculated by dividing the volume of the outside dimensions of the chamber load by the volume of the inside dimensions of the chamber then multiplying the product by 100. Load factor (v/v) was 41% or 44% of the chamber volume for EPS and TKV box types, respectively. Both box types contained the same amount of table grapes and the load factor was considered to be equivalent for both test series. Results from Series I tests (Table 1; Fig. 1) with EPS boxes showed MB concentrations to be higher at the start and lower at the end (2 h) of the exposure period due to higher displacement and sorptive properties of the EPS material. However, the see-saw gas concentrations resulted in C × T products that were actually higher with EPS compared to TKV boxes.

MB doses in Series II tests were increased proportionately, based on end readings observed in Series I tests, to attempt to obtain comparable end readings between boxes and equal to or above those observed in Series I tests for the TKV boxes. Therefore, MB dose for EPS boxes was increased by 8 g/m^3 (from 32 to 40 g/m^3) at 21 °C and by 4 g/m^3 for the 48 g/m^3 schedule for a dose of 52 g/m^3 at 10 °C. Results from Series II tests are shown in Table 2 and Fig. 2. Comparison of data for EPS and TKV boxes showed significantly higher gas concentrations at all sample times and much higher C × T products between treatments for the EPS box, with only one exception. There was no difference in gas concentrations at the end of the exposure period between TKV and EPS boxes at 10 °C when fumigated with 48 or 52 g/m^3, respectively. An adjustment of just 4 g/m^3 more MB

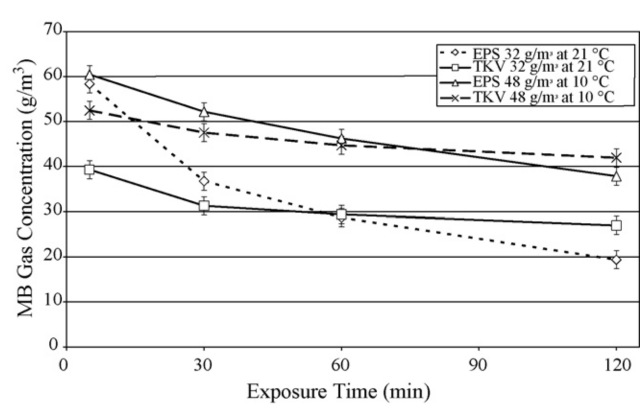

Fig. 1. MB gas concentrations from table grapes exposed to mealybug treatment schedules: 32 g/m^3 at 21 °C or 48 g/m^3 at 10 °C EPS vs. TKV shipping boxes.

Table 2
Series II comparisons: MB gas concentrations, sorption, and C × T products from modified mealybug treatment schedule: MB dose increased to 40 g/m^3 at 21 °C and to 52 g/m^3 at 10 °C to compensate for sorption of MB by EPS boxes

Box type	MB dose (g/m^3)	Sample (n)	MB gas concentration (g/m^3; mean ± S.D.) over time (min)				% Sorption	C × T product (g h m^{-3})
			Start (5)	30	60	120		
2 h at 21 °C								
EPS	40	Air	47.9 ± 0.2	42.8 ± 0.3	38.1 ± 0.3	32.3 ± 0.3	32.6 ± 0.7	78.1 ± 0.4
		Box	47.1 ± 0.1	42.6 ± 0.2	38.0 ± 0.5	32.2 ± 0.3	NA	77.7 ± 0.6
TKV	32	Air	34.4 ± 0.5	31.7 ± 0.4	29.9 ± 0.4	27.9 ± 0.5	19.0 ± 1.0	60.9 ± 0.8
		Box	32.7 ± 0.9	31.4 ± 0.2	29.5 ± 0.3	27.7 ± 0.4	NA	59.9 ± 0.6
$P_{0.05}$[a]			>0.0001	>0.0001	>0.0001	>0.0001	>0.0001	>0.0001
2 h at 10 °C								
EPS	52	Air	62.3 ± 0.5	54.7 ± 2.1	49.3 ± 1.4	41.4 ± 1.2	33.5 ± 1.8	100.6 ± 2.7
		Box	59.8 ± 1.6	54.4 ± 1.7	49.3 ± 1.2	41.5 ± 1.0	NA	99.9 ± 2.6
TKV	48	Air	50.5 ± 0.9	46.0 ± 0.4	43.9 ± 0.7	41.2 ± 0.6	18.3 ± 1.8	89.2 ± 1.1
		Box	47.7 ± 3.1	45.4 ± 0.8	43.4 ± 0.9	40.8 ± 0.8	NA	87.6 ± 2.3
$P_{0.05}$[a]			>0.0001	>0.0001	>0.0001	NS	>0.001	>0.0001

[a] ANOVA: differences between EPS and TKV boxes, within treatments, are not significant if $P > 0.05$ or significant if $P < 0.05$ (SAS, 2002–2003).

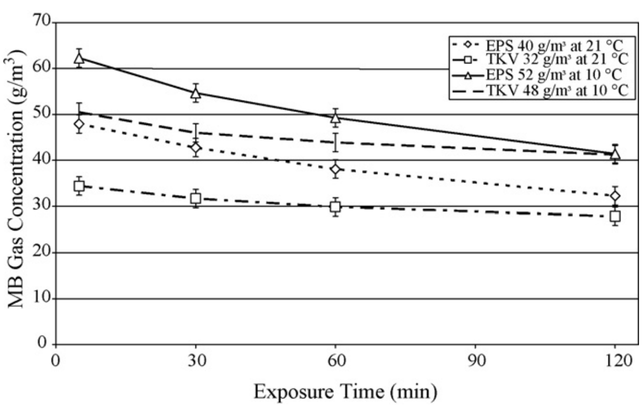

Fig. 2. MB gas concentrations from table grapes exposed to modified treatment schedules: dose adjusted to obtain equivalence of end reading of MB gas EPS vs. TKV shipping boxes.

for EPS boxes produced the end reading that corresponded to that in the TKV boxes. Increasing dose of MB by 8–40 g/m^3 at 21 °C for the EPS box was too much and resulted in a significantly higher end reading compared to the TKV box and treatment. The data suggests that an adjustment of just 4 g/m^3 more (from 32 to 36 g/m^3) for EPS boxes would have been adequate. Certainly it is important to avoid increasing the dose of MB by more than is necessary to achieve the same end reading as found in the TKV boxes. Concentrations of MB gas outside (air) or inside (box) the boxes was not significantly different ($P > 0.05$), except for a few start readings only at 21 °C. The data showed that distribution of MB gas to the grapes packed inside either box type was not hindered, slowed, or reduced.

3.2. Phytotoxicity

Results from phytotoxicity evaluations are shown in Table 3. There was no significant difference in any quality parameter associated with package type. The most important table grape quality loss often associated with MB fumigation, berry browning, occurred at a low frequency and was not associated with MB dosage. The only significant elevation in berry browning occurred in Series II in EPS boxes fumigated with a MB C × T product of 100.6 g h m^{-3}. These berries were also among the oldest at harvest and the last evaluated, so the browning may have been a consequence of MB dosage or their age, and it may have been influenced by the high

Table 3
Quality of ‘Crimson seedless’ table grapes after exposure to MB gas fumigation for 2 h followed by storage for 3–4 weeks at 1 °C and 2 d at 20 °C[a,b]

Temperature (°C)	MB dose (g/m^3)	Box type	C × T product (g h m^{-3})	Quality				
				Overall appearance[c]	Rachis appearance[d]	Shatter[e] (%)	Decay[e] (%)	Internal browning[e] (%)
Series I								
21	32	EPS	62.7 a	2.5 a	3.2 a	22.1 b	9.6 a	0.0 a
		TKV	59.7 a	2.6 a	3.2 a	17.7 ab	12.8 ab	0.3 a
10	48	EPS	92.6 c	2.6 a	3.2 a	13.7 a	11.5 ab	0.3 a
		TKV	89.4 c	2.5 a	3.1 a	12.9 a	10.2 ab	0.6 a
Series II								
21	40	EPS	78.1 b	3.5 bc	2.7 b	29.4 c	34.9 c	0.4 a
	32	TKV	60.9 a	3.6 bc	2.3 c	38.7 d	47.6 d	1.1 ab
10	52	EPS	100.6 d	3.8 cd	2.3 c	49.3 e	65.3 e	1.7 b
	48	TKV	99.9 d	4.0 d	2.2 c	62.1 f	69.5 e	0.0 a
Controls								
	0	EPS	0	3.3 bc	2.2 c	22.1 bc	25.0 bc	0.0 a
	0	TKV	0	3.2 b	3.1 a	22.6 bc	25.5 bc	0.0 a

[a] The grapes originated from the same vineyard, but their dates of harvest differed. Series I, control, and Series II grapes were harvested late September, early October, and late October, respectively.
[b] ANOVA: Same or different letter designations indicate differences between means are not significant ($P > 0.05$) or significant ($P < 0.05$), respectively (SAS, 2002–2003).
[c] Overall appearance was rated on a scale of 1 (excellent), 2 (good), 3 (fair but acceptable), or 4 (poor and unacceptable).
[d] Rachis appearance was rated on a scale of 1 (fresh and green), 2 (about half green), 3 (some green color present), or 4 (completely brown).
[e] Shatter, decay, and internal browning are the percent of detached, decayed, or internally brown berries, respectively.

incidence of decay that was present among these oldest berries. Other quality parameters were not high and declined primarily as a result of the condition of the berries at harvest, lack of disease control treatments during storage, and drying of the cluster rachis. The quality of the grapes significantly declined between Series I and II, because the Series II berries were older at the time of harvest which was later in the season and their quality had declined naturally.

4. Discussion

4.1. Fumigation

Data from Series I tests indicated an increase of MB dose from 32 to 40 g/m3 for EPS boxes fumigated at 21 °C. However, this increase of 8 g/m^3 was an over-adjustment and the data supported an increase of just 4 g/m^3 was needed for EPS boxes for all MB doses, regardless of temperature regimen. Data also showed significant differences for EPS boxes compared to TKV boxes with higher gas concentrations at the start (higher displacement by EPS boxes), lower gas concentrations at the end (higher sorption from EPS boxes), but because MB gas was higher for about the first half of the exposure test and lower the second half, the C × T products were the same or higher inside EPS compared to TKV boxes, when fumigated with the current MB schedule.

4.2. Phytotoxicity

Observations of fruit quality in this work indicate that MB at the rates evaluated will not be harmful. The primary injury caused by MB to table grapes is internal browning (Auda et al., 1977; Nelson and Spitler, 1982). Browning is primarily of concern in green cultivars, where it is readily apparent to consumers. Phillips et al. (1984) reported 32 g/m^3 MB for 3.5 h did not cause internal browning or other injuries to table grapes. Previously, we found the quality of the popular table grape varieties ‘Red Globe’, ‘Prima Red’, and ‘Ruby Seedless’ were unharmed by fumigation with 64 g/m^3 of MB for 2 h, where C × T products exceeded 100 ppm h (Smilanick et al., 2000). However, Nelson and Spitler (1982) reported that this rate increased the incidence of internal browning of ‘Thompson Seedless’ berries when fumigation was >4 h duration. Liyanage et al. (1993) showed glutathione, a natural antioxidant, content was greatly reduced by MB fumigation and its reduction could lead to berry browning, although they did not demonstrate a causative relationship between the decline in glutathione reduction and internal browning.

5. Conclusion

MB gas concentration data reported herein indicate that either box type, TKV or EPS, can be used for shipping table grapes and still provide an efficacious treatment based on MB gas concentrations with no or minor adjustments to the AQIS approved treatment schedules. First, if exposure to MB gas is based on C × T product (the area under the exposure curve expressed in g h m^{-3}), then no significant difference was observed between box types and no adjustment in MB dose is necessary regardless of box type used for shipping table grapes, TKV or EPS. Several studies have shown that C × T is linearly correlated to insect mortality when using MB (Estes, 1965; Bell, 1977, 1978; Bond, 1984). Thus if the C × T product used to give 100% mortality of the mealybugs is established, then, if that C × T is achieved, the same result should occur. Second, if minimum exposure is based on the final or ending (in this case 2 h reading), then a small adjustment is needed when EPS boxes are used. The data supports and we would recommend that the MB treatments currently required by the AQIS (2004) Work Plan be increased by just 4 g/m^3 for EPS boxes for each temperature regimen. If these recommendations are followed we feel there is no reason that EPS boxes could not also be in addition to the TKV box for shipping table grapes to markets requiring a pre-shipment MB treatment. Historically, however, equivalence of effective dosages between treatment schedules has often been based on equivalency of C × T product exposure (Monro, 1969). If C × T product was used as the criteria for determining exposure to MB then no adjustment to the treatment schedules would be necessary, because C × T product was equal to or even greater when table grapes were packed and fumigated in EPS boxes compared to TKV boxes. Furthermore, we would not expect any of these MB schedules to cause undue injury to the table grapes. Also, it is likely that EPS boxes could be used in lieu of TKV boxes for other commodities as well, although we did not include other commodities in this series.

Acknowledgements

We thank Leigh Schmidt, Monir Mansour, and Jenny Ghosoph for conducting the quality examinations, and the California Table Grape Commission who partially funded this research.

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